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Bioinformatic Analysis of the Human μ Opioid Receptor (OPRM1) Splice and Polymorphic Variants

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ABSTRACT Mu opioid receptor (OPRM1), a member of the G-protein coupled receptor superfamily, mediates the analgesic and euphoric effects of opioid drugs. The sequences of OPRM1 cDNA and reported splice variants were used to search the public and Celera genomic databases. The matched sequences were analyzed to assemble an OPRM1 genomic contig. Human OPRM1 gene was estimated to span at least 90 kb in the chromosome 6q24-25 region. Four coding exons are separated by 3 introns. While intron 2 has only 773 bp, these databases for the first time provide the precise length of and other information about long introns 1 and 3, containing 50 and 27 kb, respectively. When a consensus exon/intron splice junction at the end of the coding exon 3 was not utilized, it may have resulted in continuous translation of the exon to yield the splice variant OPRM1A. The study did not identify human orthologs of other OPRM1 variants that had been reported for mouse OPRM1, although several proposed exons were found to be included in mouse genomic clones. Single nucleotide polymorphisms in the OPRM1 gene were also analyzed and summarized, which could provide potential polymorphic markers for molecular genetic studies.

KEYWORDS: OPRM1, MOR1, opioid receptor, ortholog, splice, variant, polymorphism.

INTRODUCTION Opioids (prototype: morphine) are primarily used as analgesics, although they have other pharmacological actions and are used to treat cough, diarrhea, and other conditions. These effects are mediated by opioid receptors. Three opioid receptors, μ, Î´, and Îº, have been identified by receptor binding, [1-3] pharmacological methods (see reviews, eg, Smith and Loh [4] and Lewis et al, [5] and molecular cloning. [6-11] The μ opioid receptor (OPRM1) is the primary receptor mediating the analgesic and rewarding effects of opioids, and thus it has been the focus of intensive research, with the hope of finding more potent analgesics with minimal undesired actions. Repeated use of opioids often leads to the development of tolerance of these drugs, which greatly limits the effectiveness of pain control. In addition, drug dependence on opioids remains a medical and social problem. Large individual differences have been observed in the levels of brain OPRM1, [12] pain response, [13-15] response to opioid analgesia, [16-18] and vulnerability to drug addiction, [15,19] and possibly the kinetics and severity of opioid tolerance. [20] Explanations of some of these differences will likely come from the studies of the genes involved. Pharmacological and genetic studies suggest that OPRM1 may be such a candidate gene. [15,21]

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The human OPRM1 complementary DNA (DNA), also abbreviated MOR1, was cloned independently by different groups. [22,23] However, the exact genomic arrangement of OPRM1 remained unclear largely because of its long introns. [15] Using the human genome sequences available from the Human Genome Project and Celera database, [24,25] we seek to assemble the OPRM1 genomic structure by identifying the chromosome locations of the gene and the exon and intron assignment and junctions.

Shortly after the cloning of OPRM1 cDNA, 2 splice variants were identified from human [26] and mouse [27] cell lines by reverse transcription-polymerase chain reaction (RT-PCR). Multiple subtypes of OPRM1 have previously been reported and characterized pharmacologically [28-31]; therefore, splice variants could provide genetic evidence for receptor subtypes. Recently, another research group reported the discovery of 4 additional C-terminus variants of mouse OPRM1 using the rapid amplification of cDNA end and RT-PCR methods; however, no clones have been directly isolated from cDNA libraries. [32,33] Just before the submission of this article, the same group further identified several N-terminal splice variants of mouse OPRM1 using similar approaches. [34] The current study attempts to map the locations of potential human orthologs of these variants in relation to that of the OPRM1 gene.

Another aim of this study is to analyze and determine the frequencies of the single nucleotide polymorphisms (SNPs) that have been reported in molecular genetic studies and sequencing efforts.

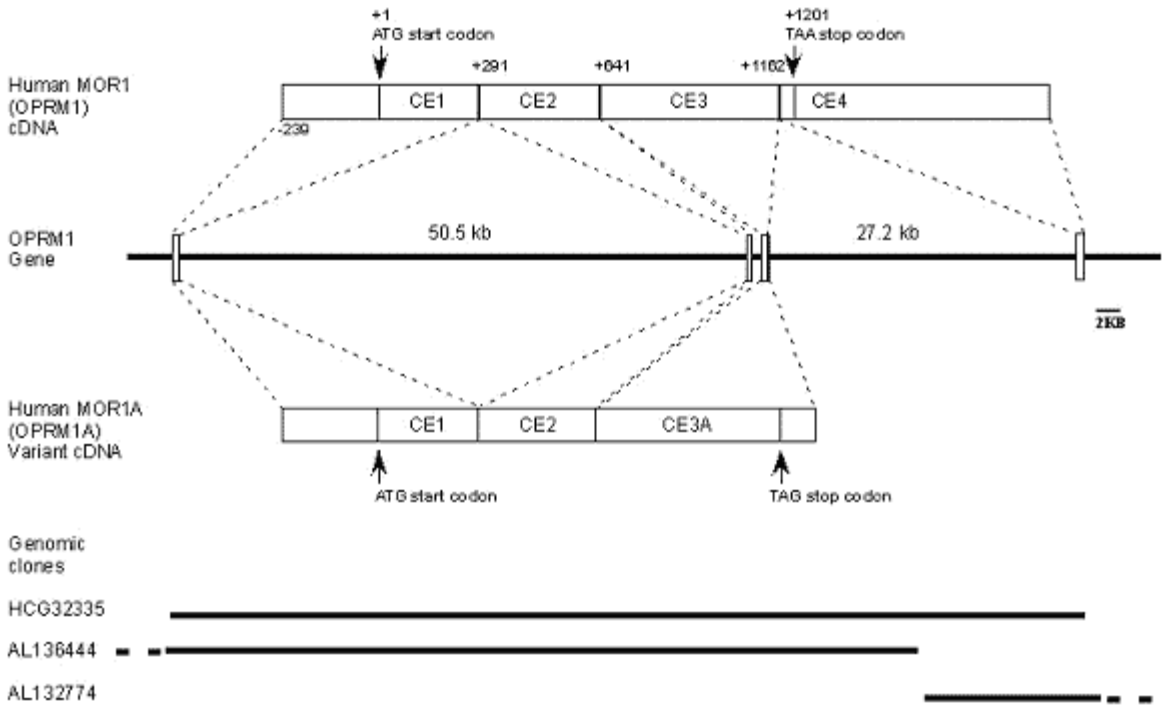

Figure 1. Genomic structure of human m opioid receptor (OPRM1) gene.
Coding exons (CEs) are separated by 3 introns to occupy over 80 kb genomic sequence in the chromosome 6q24-25 region (position: 161 380 kb-161 460 kb). The sizes of these 3 introns are 50 461, 773, and 27 207 bp, respectively. AL136444 extends 33 kb 5' of CE1. AL132774 contains 95-kb sequence beyond the translation stop. Only 3 coding exons are used in the splice variant OPRM1.

MATERIALS AND METHODS The sequences of the cloned human OPRM1 (Appendix 1) and reported splice variants (Appendix 2) were used to search GenBank (Release 124.0, June 2001,) and the Celera database ( ) using the Blastn program. [35] A BLOSUM-62 matrix with default parameters was used for most analyses. Sequences matches were further analyzed by BESTFIT and FILEUP programs using the GCG package (Wisconsin Package Version 10.2, Genetics Computer Group, Madison, WI). From these analyses, a human OPRM1 contig was constructed based on the consensus sequence. This contig was then used to search for a variable number of tandem repeats using the COMPARE and DOTPLOT programs. Mouse OPRM1 variants were analyzed in a similar fashion except that all available sequences were used for comparisons with human EST, transcripts, and genomic sequences deposited in GenBank and the Celera database. For those sequences that failed to find significant homology in the human genome, we further used them and their available intron sequences to search GenBank for all available sequences, including EST, unfinished high-throughput genomic sequences, genome survey sequence databases, and sequences deposited within the past month.

RESULTS Human OPRM1 cDNA sequence

Three human OPRM1 cDNA sequences have been published and deposited in GenBank (L25119, L29301, and U12569). In addition, a number of sequences were recorded via direct submission by sequencing laboratories (eg, NT_02345, XM_004341). These sequences were compared using BESTFIT and PILEUP programs to construct a consensus OPRM1 cDNA sequence (Appendix 1). The longest accountable human OPRM1 cDNA is 2187 bp in length, of which 1203 bp are used to encode 400 amino acid (aa) residues and the stop codon (**Figure 1**). To identify the sequence location, we assign the "+1" for the first nucleotide of the

Table 1. Human OPRM1 Coding Exon Arrangement*

No	5' Junction (Sequence at 3' Splice Site)	Total Size (bp)	Coding Size (bp)	Receptor Regions	3' Junction (Sequence at 5' Splice Site)
1	TBD	527	290	N-terminus, TM1	ACTTCCTGGTCATGTATGTGATTGTC**AG**
2	ATACACCAAGATGAAGACTGCCACCAACATCT	353	353	i1, TM2-4, part of e2	TCTTCCTGTAATGTTCATGGCTACAACAAAATA CAGGCA**AG**
3	GTTCCATAGATTGTACACTAACATTCTCTCATC CAACCTGGTACTGGGAAAACCTGCTG	521	521	e2, TM5-7, C-terminus	CACCCCTCCACGGCCAATACAGTGGATAGAAC TAATCATC**AG**
4	CTAGAAAATCTGGAAGCAGAAACTGCTCCGTT GCCCTA	786	39	C-terminus last 13 aa	TBD

*aa indicates amino acid; e2, extracellular loop 2; i1, intracellular loop 1; OPRM1, μ opioid receptor; TBD, to be determined; and TM, transmembrane domain. Consensus AG/GT sequences at 5' splice sites, and AG/NN at the 3' splice sites are in bold.

Table 2. Human OPRM1 Gene Intron Arrangement*

No	5' Junction (Sequence at 5' Splice Site)	Size (bp)	3' Junction (Sequence at 3' Splice Site)
1	**GT**AAGGAAAGCGCCAGGGCTCCGAGCGGAGG G	50,461	TTTTACTGATTCTCACTCTTCTTCCTTTATCTCCT**AG**
2	**GT**GAGTGATGTTACCAGCCTGAGGGAAGGAG G	773	TTGCTGCTAATTTTTCCTTTAAATTCCTTTCTTCT**AG**
3	**GT**ACGCAGTCTCTAGAATTAGGTATATCTACTG	27,207	GAAATGTTCACTGTCTTTGCTCTTTCTCTCCTTTC**AG**

*OPRM1 indicates ? opioid receptor. Consensus AG/GT sequences at 5' splice sites, and AG/NN at the 3' splice sites are in bold.

translation initiation codon (ATG). Therefore, a negative number indicates the location 5' (upstream) to the translation start.

OPRM1 genomic sequence

The OPRM1 cDNA sequence was then used to search the genomic databases. A chromosome 6 clone, HCG32335 (Celera), was found to have significant nucleotide identity with the cDNA probe. This clone has perfect matches with the OPRM1 cDNA in 4 segments, corresponding to the 4 coding exons (**Figure 1**). Exon 1 has a 290-bp coding sequence encoding for 97 aa residues at the extracellular N-terminus and transmembrane domain (TM) 1 (**Tables 1** and **2**). Following coding exon 1 is a very large intron 1 of 50 461 bp in length. Coding exon 2 includes a sequence of 118 aa that make up the intracellular loop 1 to TM4 and part of the extracellular loop 2. Intron 2 contains 773 bp and is followed by exon 3. One hundred and seventy-four aa are encoded by exon 3, covering the regions including part of extracellular loop 2, TM5-TM7, and part of intracellular C-terminus. Before the short exon 4, responsible for 12 aa, is another long intron 3 of 27 207 bp. All exon-intron junctions match the consensus splicing requirements (**Tables 1** and **2**).

Adding up these exons and introns, OPRM1 is over 80 kb in chromosome 6 (161 380 kb-161 460 kb), which is entirely covered by the clone HCG32335. At 80 591 bp in length, HCG32335 clone, however, does not provide much sequence information about the 5' UTR region, including the promoter regions. [36,37] The search in GenBank turned up 2 clones: AL136444 and AL132774. AL136444 was deposited in the antisense direction; the reverse complementary sequence of AL136444 overlapped with HCG32335 for 63 620 bp. AL136444 also contained OPRM1 exons 1, 2, and 3 and part of intron 3. In addition, AL136444 included 32 690 bp 5' to the OPRM1 cDNA and HCG32335 (Appendix 1). OPRM1 exon 4 was found in another chromosome 6 clone, AL132774, which has an additional 95 kb beyond the OPRM1 translation stop codon. These 3 clones allow us to assemble a 200-kb OPRM1 contig (**Figure 1**); the overall sequence agreement in the overlapped regions was over 99%.

OPMR1 alternative splicing variants

Shortly after the cloning of the human OPRM1, a 3' splicing variant was reported and termed OPRM1A. [26] OPRM1A lacked the last 12 aa found in OPRM1. When the OPRM1A 3' unique sequence was used to search genome databases, it was found to be 100% identical with a region in the genomic clones HCG32335 and AL136444 (**Table 3**). The sequence alignment suggested that OPRM1A has the same coding exons 1

Table 3. Summary of the Proposed OPRM1 Exons*

	Human Homologous Sequences (Homology)	Mouse Homologous Sequences (Homology)	Comments
CE1 (527 bp)†	AL136444, HCG32335	eg, U10558 (78%)	
CE2 (353 bp)	AL136444, HCG32335	eg, U10559 (90%)	
CE3 (521 bp)	AL136444, HCG32335	eg, U10560 (86%)	
CE4 (786 bp)	AL136444, HCG32335	eg, U10561 (84%)	84% similarity in the first 110 bp
E3A (189 bp)‡	AL136444, HCG32335	AF167565§	
E5 (280 bp)⁇	NF	AC055776 (85%)	AC055776: unfinished mouse chromosome 10 clone
E6 (121 bp)¶	NF	NF	
E7 (89 bp)¶	AJ310556, AL445220 (88%)	AC101798 (100%)	AJ310556: human PIP3-E gene AC101798: mouse working genomic sequence draft
E8 (66 bp)¶	NF	AZ365875 (100%)	AZ365875: mouse GSS*; significant identity to a segment in AC101798
E9 (389 bp)¶	NF	AC091451 (100%)	AC091451: mouse chromosome X PAC clone
E10 (186 bp)**	NF	AC055776 (97%)	
E11 (185 bp)††	NF	AC101942 (100%)	AC101942: significant identity to AL136444
E12 (129 bp)††	NF	BH121554 (99%)	BH121554: mouse GSS; no identity to AC101942
E13 (149 bp)††	NF	AC055776 (97%)	
E14 (108 bp)	NF	AC055776 (100%)	

*GSS indicates genome survey sequence; NF, no significantly homologous sequence found; OPRM1, ? opioid receptor; and PIP3-E, phosphoinositide-binding protein, PAC clone, P1 artificial chromosome clone.
†CE: human OPRM1 coding exons. CE1-4 are included to show the overall homology between mouse and human OPRM1.
‡E3A: human OPRM1A third coding exon, which was initially reported by Bare and colleagues (26).
§AF167565: reported mouse OPRM1A sequence. Overall, AF167565 shares 86% nucleotide identity with human OPRM1A. However, most similarity is due to the shared core sequence of OPRM1. Mouse OPRM1A differs from the human ortholog by the last 3 amino acids. There is no homology in the 3'UTR between human and mouse OPRM1A either.
⁇E5 was first reported by Zimprich and colleagues (27).
¶E6 to E9 were reported by Pan and colleagues (32).
**E10 was reported by Pan and colleagues (33).
††E11 to E14 were reported by Pan and colleagues (34).

and 2, and introns 1 and 2, as OPRM1. OPRM1A might have been formed when the consensus splice site AG/GT at the end of OPRM1 coding exon 3 was not used. Instead, it continued to transcribe, forming its own third coding exon (CE3A), which included 15 additional bp and encoded for 4 aa (V-R-S-L) before the stop codon TAG. All known 98-bp 3'UTR sequences [26] were found to follow immediately after the stop codon in the genomic clones (**Figure 1**).

At least 5 additional 3' and 8 5' splice variants have been proposed; however, no human orthologs are known. Since the exact exon numbers are not clear for OPRM1 at this point, we propose using coding exons for the currently known 4 exons. Other proposed exons are referred to as E5 to E14, keeping the same numerical numbers as were initially proposed. [32-34] We first sought to identify these alternative variant exons, if any, in the human genome. For those sequences that failed to find significant homology in the human genome, we further used them and their available intron sequences to search GenBank for all available sequences. This approach yielded genomic structures of several proposed mouse exons.

MOR1B was initially reported for rat OPRM1. [27] It differed from OPRM1 by the last 12 aa, indicating the absence of coding exon 4. Instead, another exon (E5, Appendix 2) replaced the coding exon 4 and encoded 4 alternative aa (K-D-L-F). [27] The rat MOR1B was 91% homologous with a mouse sequence (AF167566) in a smaller region (125 bp). E5 (274 bp) was found to share 85% sequence identity with a sequence (AC055776) from the unfinished mouse chromosome 10 clone RP23-11015 (**Table 3**). Unfortunately, AC055776 was still a working draft made up of 21 sequence contigs in no particular order. Analysis of AC055776 indicated that it contained all 4 mouse OPRM1 coding exons. [38] E10 was also found to be included in AC055776. However, E5 and E10 were found in separate contigs and not located near any of the coding exons; therefore, we were not able to estimate the distance between these exons.

Four other mouse OPRM1 3' variants require the use of 4 additional exons (E6-E9, see Appendix 2). These variants differ in their carboxyl terminal sequences. When expressed in Chinese hamster ovary (CHO) cells, all variants showed high affinity to OPRM1 agonists, [31,32] which was not entirely unexpected. OPRM1 lacking large parts of the intracellular C-tail sequences exhibited a nearly identical ligand binding profile. [39] Antibodies raised to recognize specific variants revealed distinct expression regions from OPRM1, and within these variants. [40-42] Three proposed variants, MOR1C, E, and F, call for the presence of exon E7. We identified the genomic arrangement of E7 since it was found to be part of an unfinished mouse clone RP24-217J7 (AC101798). Interestingly, E7 also demonstrated 88% identity with a region in human phosphoinositide-binding protein (PIP3-E) gene (AJ310556), located in human genome clone AL445220 (**Table 3**) and a Celera sequence. The full-length PIP3-E gene was found in 3 human chromosome 6 clones: AL132774, AL445220, and AL033376 spanning ~90 kb. We then compared these PIP3-E-containing human clones with the mouse working

sequence AC101798. Sequences included in AC101798 were found to have significant matches (90%-100% in some regions) with regions in these human sequences and AL136444. No other human genomic sequences have significant identity with AC101798. These comparisons strongly suggested that mouse sequence AC101798 was from the mouse chromosome 10, most likely from the OPRM1 and PIP3-E gene locus.

E8 was reported to be composed of 66 bp, which was found to be part (443-508) of the mouse 10-kb plasmid UUGC1M library clone 1M0112B22R (**Table 3**). Unfortunately, only 545 bp of the latter sequence is deposited as AZ365875. We, nevertheless, used these 545 bp to search databases for homologous sequences, and we found that the first part (1-232) was identical with a segment (51028-51260) from a contig (50541-51265) in the clone AC101798. A portion (300 bp) of the same contig was found to match a segment from the rat genomic sequence AC114432 (80% identity), but there was no homology with any known human sequences.

The entire sequence of E9 (398 bp), the last putative exon reported for the mouse OPRM1, was found to share 99% nucleotide identity with an unfinished mouse PAC clone AC091451 from the chromosome X (**Table 3**).

The 8 newly reported 5' splice variants used 4 additional exons (E11-E14, Appendix 2). Three variants (MOR1H, I, J) can produce protein sequences identical to OPRM1. Other variants produce either 6- or no transmembrane-spanning proteins. The RT-PCR method revealed varying degrees of MOR1H, I, and J in various brain regions. E11 and E12 were reported to be the two most 5'-exons that are 1.8 kb apart. [34] We found E11 to be part of the mouse genomic sequence draft AC101942. Comparison of AC101942 with the human OPRM1 sequence AL136444 yielded multiple sequence alignments that were relatively well conserved (75%-91% similarity). Genomic search using E11 found no human homologous sequence. E11 was included in a single sequence readout of 534 bp in AC101942. When these 534-bp sequences were compared with AL136444, a moderate degree (82%) of identity was found in 2 segments, 30 kb from the OPRM1 coding exon 1. However, E11 was not included in either of the homologous segments. E12 had no significant identity with any sequences in AC101942. Intron regions surrounding E11 (AF062755), but not E12, were also found to be included in AC101942. E12 was found to be part of the mouse genomic clone RPCI-24; only the 538-bp sequence was known (BH121554). When the entire 538-bp sequence of BH121554 was used to search all available databases, no additional homologous sequence was retrieved.

Two other 5' variant exons, E13 and E14, were found in mouse chromosome 10 working draft AC055776. AC055776 included all 4 coding exons, [38] E5, and E10. Interestingly, both E13 and E14 were found in the same contig (3051-5112). If this contig was indeed reliable, the distance between E13 and E14 was estimated at 1448 bp, which was significantly shorter than what had been reported. [34] The previous estimate was around 21 kb between E13 and E14. Consensus intron/exon splicing sites were met at all these proposed exons (E5, E10, E13, and E14) and their intron junctions; however, none of these exons, and hence the variants composed of them, were found to have human orthologs available. The same mouse clone AC055776, however, did not contain E12.

E6 used by the proposed variant MOR1F failed to locate any significant similarity with all sequences searched.

OPRM1 SNPs

A number of SNPs have been identified in the OPRM1 gene through direct sequencing and other methods, which have been so far largely limited to the cDNA sequence and short intron 2. [15, 19, 43-47] Ten coding region SNPs have been identified, of which 8 are nonsynonymous. Several rare SNPs in the I3 loop have been tested and shown to impair receptor signaling [46,48] ; however, the significance of these SNPs in heterozygous individuals carrying the SNPs (no homozygous case has been reported) remains to be demonstrated. Two coding region SNPs occur at relatively high frequencies, including 17 C/T and 118 A/G. The 118 A/G SNP leads to aa substitution, Asn/Asp, at the extracellular N-terminus, which can potentially eliminate a consensus N-glycosylation site. When MOR1 A118G mutation was expressed in AV12 cells, it exhibited nearly identical affinity and activation by opioid ligands, with the exception of the large endogenous peptide -endorphin. [44] A118G variant showed higher affinity and increased activity to -endorphin [44] ; however, the same results were not reproduced when A118G variant was expressed in COS-7 cells. [48] Molecular genetic studies employing polymorphism 118 A/G in drug and alcohol abuse have so far yielded conflicting results. Most studies found no significant association of 118 A/G with polysubstance abuse and alcoholism, [43,44,47,49,50] although there was a trend of increased G allele in polysubstance abusers. [47] Two studies, however, found significant association of 118 A/G with alcoholism in a mixed population sample (primarily Caucasian subjects) [51] and with opioid addiction in Chinese study subjects. [52] A list of all reported human OPRM1 SNPs appears in **Table 4** .

Table 4. SNPs in OPRM1 Gene*

Position	Gene Location	Amino Acid Change	Polymorphic Allele Frequency	References
–1045 A/G	5'UTR	—	~1.4%	(45)
–995 C/A	5'UTR	—	NA	(45)
–692 G/C	5'UTR	—	4.3%	(45)
–554 G/A	5'UTR	—	NA	(45)
–488 G/T	5'UTR	—	NA	(45)
–254 A/C	5'UTR	—	<1%	(45)
–236 A/G	5'UTR	—	<1%	(45)
–172 G/T	5'UTR	—	11.4%	(45)
–133 C/T	5'UTR	—	<1%	(45)
–111 C/T	5'UTR	—	~1.4%	(45)
–54 G/T	5'UTR	—	NA	(45)
–38 C/A	5'UTR	—	1.4%-5%	(19, 45)
17 C/T	Exon 1	6 Ala/Val	6.6%-19%	(19, 43-45)
24 G/A	Exon 1	Synonymous	~2.0%	(44, 45)
118 A/G	Exon 1	Asn/Asp	9.2%-13.9%	(43-45)
440 C/G	Exon 2	Ser/Cys	<1%	(43, 45)
454 A/G	Exon 2	152 Asn/Asp	~1.4%	
IVS2+31 G/A	Intron 2	—	14.3%	(45, 53)
IVS2+106 T/C	Intron 2	—	~1.4%	(45)
IVS2+397 T/A	Intron 2	—	~1.4%	(45)
IVS2+438 G/A	Intron 2	—	~4.3%	(45)
IVS2+480 T/C	Intron 2	—	NA	(45)
IVS2+534 C/T	Intron 2	—	<1%	(45)
IVS2+691 C/G	Intron 2	—	40%-45%	(43, 45)
779 G/A	Exon 3	260 Arg/His	<1%	(44, 45)
794 G/A	Exon 3	265 Arg/His	<1%	(45, 46)
802 T/C	Exon 3	268 Ser/Pro	<1%	(45)
820 G/A	Exon 3	274 Asp/Asn	<1%	(45, 46)
942 G/A	Exon 3	Synonymous	<1%	(44, 45)
/S3+37 A/C	Intron 3	—	NA	(45)
401 G/C	3'UTR	—	~2.9%	(45)

*NA indicates not applicable (SNPs with no reliable allele frequencies); OPRM1, mu opioid receptor; SNP, single nucleotide polymorphism.

DISCUSSION In this study we analyzed human OPRM1 sequences and identified ~2.2 kb of accountable cDNA sequence. Considering that human OPRM1 mRNA is over 12 kb ([32, 37,] and Wang et al, unpublished results), there are additional cDNA sequences at either the 5' or the 3' end. The available human genomic data for the first time allowed a clear arrangement of the OPR M1 gene, with precise intron length and intron/exon junctions. Adding together the unaccountable ~10 kb cDNA and ~80 kb genomic sequences that 4 coding exons and introns occupy, OPRM1 is at least 90 kb. This number may be substantially longer if there are additional exons and introns to make up the ~10-kb additional cDNA sequence.

Human OPRM1A variant appears to be valid, given that the entire OPRM1A variant sequence can be found in intron 3 of OPRM1. However, this variant does not provide any cDNA usage information beyond what has been identified for OPRM1.

On the contrary, the other reported 5' and 3' splice variants [27, 32-34] may offer insights into additional cDNA sequence usage as well as OPRM1 multiple subtypes. However, the only human homologue of these additional exons identified in the study was E7, which may represent a sequence from the human PIP3-E gene (AJ310556). PIP3-E, previously identified as KIAA0403, is the first gene 3' from OPRM1 with known function. [54] Another gene, LOC154045 (XM 094669), was identified by only a computer predication program, GenomeScan, and located between OPRM1 and PIP3-E. Most likely, E7 also represents part of mouse PIP3-E, although such information is not available from the mouse genome. E7 was used in variant MOR1C, E, F, and M. [32-34] E9, which was required for MOR1C, D, E, F, M, and N, was found to have 100% identity with mouse chromosome X sequence (AC091451). Due to the draft nature of AC091451, it cannot be entirely ruled out that the E9-containing portion of AC091451 has sequencing or cloning errors. Antibodies made from the unique peptide sequences of MOR1C and MOR1D have been used to detect immunoactivities for these proposed variants in rat and mouse. [40,41] In both cases, the antibodies were

made from peptide sequences that were primarily encoded by E8, so the immunoreactivity was largely independent of the presence of E7 or E9. Our analysis indicated that E8 was located in the mouse sequence AC101798, a mouse chromosome 10 clone that also included OPRM1. In addition, part of a larger sequence containing E8 was found to have a rat homologue. There has been 1 study that found MOR1C-like immunoreactivity in human spinal tissue using the same MOR1C antibody. [42] However, the epitope peptide was found to have no similarity to any known human protein sequences.

The 4 mouse coding exons, and E5, 10, 13, and 14, were found in the mouse genomic clone AC055776. The distance between E13 and E14 was estimated at 1.5 kb. A segment of a sequence contig containing E14, but not the E14 region, demonstrated significant similarity to human chromosome 6 clone AL136444. These data suggest that additional exons in the 5' variant MOR1G-L and 3' variant MOR1B are within a reasonable distance from the other coding exons in OPRM1. However, we found no human ortholog to support the presence of such variants in the human genome. This is not likely to be due to the lack of human genomic sequence information, as the regions surrounding OPRM1 are well covered with overlapping sequences; it may be due instead to the differences between the 2 genomes. Although the OPRM1 nucleotide sequence is relatively well conserved (**Table 3**), lower similarity was observed in OPRM1A unique 3' sequence and 3'UTR regions between the 2 species. However, the significance and function of the OPRM1 5' variants MOR1G-L are somewhat unclear. MOR1G-L produce proteins containing 6 or 0 TMs, uncharacteristic of a G-protein coupled receptor. When alternative translational start points are used by MOR1H, I and J, a protein identical to that encoded by OPRM1 cDNA is produced in all 3 variants. If this is the case, then the OPRM1 expression is under the control of multiple promoters.

In summary, this study was able to locate the human genomic sequence for the OPRM1 variant OPRM1A. We were able to identify the mouse genomic structures for several proposed variant exons. Little evidence was uncovered to support human orthologs of 5' variants and OPRM1B, whereas similarity to human PIP3-E gene and mouse chromosome X segment raised concern for other 3' OPRM1 variants.

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